

What is claimed is:

1. A method for screening for a ligand binding protein of interest, comprising,
  - a) encapsulating one or more members of a population of cells suspected of expressing a ligand binding protein of interest in a capsule comprising permeable walls, said walls containing a first capture reagent for said ligand binding protein of interest;
  - 5 b) incubating said encapsulated cells under conditions that allow for expression of said ligand binding protein of interest and capture of said ligand binding protein by said first capture reagent;
  - c) contacting said permeable capsule with a ligand specific for said captured ligand binding protein, to form a captured protein-ligand complex, wherein said ligand
  - 10 can be the same as or different from said capture reagent;
  - d) contacting said captured protein-ligand complex with a first detection molecule that binds said protein-ligand complex to form a protein-ligand-first detection molecule complex;
  - e) contacting said protein-ligand-first detection molecule complex with a second
  - 15 detection molecule that binds said first detection molecule to form a protein-ligand-first detection molecule-second detection molecule complex;
  - f) contacting said protein-ligand-first detection molecule-second detection molecule complex with a third detection molecule comprising a detectable label that binds to said protein-ligand-first detection molecule-second detection molecule complex
  - 20 to form a protein-ligand-first detection molecule-second detection molecule-third detection molecule complex; and
  - g) detecting the presence of said detectable label bound to said capsule, thereby identifying cells in said capsule expressing said ligand binding protein of interest.
2. The method of claim 1, wherein said detecting the presence of the detectable label is by flow cytometry.

3. The method of claim 1, wherein said detecting the presence of the detectable label is by fluorescence activated cell sorting (FACS).
4. The method of claim 3, wherein said detectable label is a fluorescent label
5. The method of claim 1, further comprising recovering the cells expressing the ligand binding protein of interest from the capsule and repeating a) through g) at least once.
6. The method of claim 1, wherein said permeable capsule is a gel micro drop (GMD).
7. The method of claim 1, wherein said population of cells is selected from the group consisting of bacterial cells, yeast cells, fungal cells, insect cells, plant cells and animal cells.
8. The method of claim 1, wherein said population of cells is E. coli.
9. The method of claim 1, wherein said ligand further comprises a first binding moiety and said first detection molecule binds to said first binding moiety.
10. The method of claim 9, wherein said second detection molecule further comprises a second binding moiety.
11. The method of claim 10, wherein said third detection molecule binds to said second binding moiety.
12. The method of claim 10, wherein said second and first binding moieties are the same.

13. The method of claim 1, wherein said ligand binding protein is a receptor or an enzyme.
14. The method of claim 1, wherein said ligand binding protein is an antibody or a functional fragment thereof.
15. The method of claim 14, wherein said ligand binding protein is an Fab antibody fragment.
16. The method of claim 1, wherein said capture reagent is an antibody.
17. The method of claim 15, wherein said capture reagent is an anti-Fab antibody.
18. The method of claim 13, wherein said said ligand is an enzyme substrate or an receptor ligand.
19. The method of claim 14, wherein said ligand is an antigen.
20. The method of claim 9, wherein said first binding moiety is digoxigenin.
21. The method of claim 10, wherein said second binding moiety is digoxigenin.
22. The method of claim 1, wherein said detection molecules are antibodies.
23. The method of claim 1, further comprising
  - h) isolating the cells identified in g); placing cells from different capsules in different locations on a first permeable solid substrate and growing said cells under conditions that allow expression of the ligand binding protein of interest;
  - 5 i) contacting said first solid substrate with a second permeable solid substrate for a time sufficient to allow said ligand binding protein to diffuse from said first substrate to

said second substrate, said second solid substrate comprising a second capture reagent that binds said ligand binding protein of interest;

- 10        j) contacting the second solid substrate with a ligand for said ligand binding protein, said ligand comprising a detectable marker;
- k) detecting the presence and location of said detectable marker on said second substrate; and
- l) identifying the cells on said first substrate expressing said ligand binding protein of interest.

24.     The method of claim 23, wherein said first and second substrates are permeable membranes.

25.     The method of claim 23, wherein said second capture reagent is different from said first capture reagent.

26.     The method of claim 23, wherein said second capture reagent is an anti-Fab antibody.

27.     The method of claim 23 further comprising, isolating the cells identified in l) and repeating a) through l) at least once.

28.     The method of claim 23, further comprising isolating cells identified in l); growing said cells under conditions that allow for expression of said ligand binding protein of interest; and determining the expression of said ligand binding protein of interest by an enzyme-linked immunosorbent assay (ELISA).

29.     The method of claim 27, further comprising isolating cells identified in the last repetition of l); growing said cells under conditions that allow for expression of said ligand binding protein of interest; and determining the expression of said ligand binding protein of interest by an enzyme-linked immunosorbent assay (ELISA).

30. A method for screening for an Fab antibody fragment of interest, comprising,
- a) encapsulating one or more members of a population of cells suspected of expressing an Fab fragment of interest in a capsule comprising permeable walls, said walls containing a first capture reagent for said Fab antibody fragment of interest,
  - 5 wherein said capture reagent does not prevent said Fab fragment from interacting with its antigen;
  - b) incubating said encapsulated cells under conditions that allow for expression of said Fab fragment of interest and capture of said Fab fragment by said first capture reagent;
  - 10 c) contacting said permeable capsule with a digoxigenin labeled antigen specific for said captured Fab fragment, to form a captured Fab-antigen complex;
  - d) contacting said captured Fab-antigen complex with an anti-digoxigenin IgG that binds said Fab-antigen complex to form an Fab-antigen-anti-digoxigenin IgG complex;
  - 15 e) contacting the complex of d) with an digoxigenin labeled anti-IgG antibody to form an Fab-antigen-anti digoxigenin IgG-anti IgG antibody complex;
  - f) contacting the complex of e) with an anti digoxigenin antibody comprising a detectable label that specifically binds to said Fab-antigen-anti digoxigenin IgG-anti IgG antibody complex to form an Fab-antigen-anti digoxigenin IgG-anti IgG antibody-labeled
  - 20 anti digoxigenin antibody complex ; and
  - g) detecting the presence of said detectable label bound to said capsule, thereby identifying cells expressing the Fab fragment of interest.
  - h) isolating cells identified in g), placing cells from different capsules in different locations on a first permeable solid substrate, and growing said cells under conditions that
  - 25 allow expression of the Fab fragment of interest;
  - i) contacting said first solid substrate with a second permeable solid substrate for a time sufficient to allow said Fab fragment to diffuse from said first substrate to said second substrate, said second solid substrate comprising an anti Fab antibody that binds

the Fab fragment of interest, wherein binding of said anti Fab antibody does not prevent  
30 the Fab fragment from interacting with its antigen;

j) contacting the second solid substrate with an antigen for the Fab fragment, said antigen comprising a detectable marker;

k) detecting the presence and location of said detectable marker on said second substrate; and

35 l) identifying the cells on said first substrate expressing Fab fragment of interest using the location of said detectable marker on the second substrate;

m) repeating a) through l) at least once; and

n) isolating the cells identified in the last repetition of l); growing said cells under conditions that allow for expression of the Fab fragment of interest; and determining the  
40 expression of the Fab fragment of interest by an enzyme-linked immunosorbent assay (ELISA).

31. The method of claim 30, wherein said detecting the presence of the detectable label is by flow cytometry.

32. The method of claim 30, wherein said detecting the presence of the detectable label is by fluorescence activated cell sorting (FACS).

33. The method of claim 32, wherein said detectable label is a fluorescent label.

34. The method of claim 30, wherein said cells are selected from the group consisting of bacterial cells, fungal cells, yeast cells, insect cells, plant cells and animal cells.

35. The method of claim 30, wherein said cells are E. coli.

36. A method for screening for a ligand binding protein of interest, comprising,

- a) encapsulating one or more members of a population of cells suspected of expressing a ligand binding protein of interest in a capsule comprising permeable walls, said walls containing a first capture reagent for said ligand binding protein of interest;
- 5        b) incubating said encapsulated cells under conditions that allow for expression of said ligand binding protein of interest and capture of said ligand binding protein by said first capture reagent;
- c) contacting said permeable capsule with a ligand specific for said captured ligand binding protein, to form a captured protein-ligand complex, wherein said ligand
- 10       can be the same as or different from said capture reagent;
- d) contacting said captured protein-ligand complex with a first detection molecule that binds said protein-ligand complex to form a protein-ligand-first detection molecule complex said first detection molecule comprising an oligonucleotide;
- e) contacting said oligonucleotide with a circular polynucleotide that hybridizes to
- 15       said oligonucleotide;
- f) extending said oligonucleotide by rolling circle amplification wherein said circular polynucleotide serves as a template to produce a linear concatemer.
- g) detecting the presence of said linear concatemer bound to said capsule, thereby identifying cells in said capsule expressing said ligand binding protein of interest.
37.    The method of claim 36, further comprising hybridizing said contatemer with a detection oligonucleotide, said detection oligonucleotide comprising a detectable label.
38.    The method of claim 36, wherein said extension by rolling circle amplification uses nucleoside triphosphates comprising a detectable label.
39.    The method of claim 36, further comprising
- h) isolating cells identified in g); placing cells from different capsules in different locations on a first permeable solid substrate and growing said cells under conditions that allow expression of the ligand binding protein of interest;

- 5            i) contacting said first solid substrate with a second permeable solid substrate for a time sufficient to allow said ligand binding protein to diffuse from said first substrate to said second substrate, said second solid substrate comprising a second capture reagent that binds said ligand binding protein of interest;
- j) contacting the second solid substrate with a ligand for said ligand binding
- 10            protein, said ligand comprising a detectable marker;
- k) detecting the presence and location of said detectable marker on said second substrate; and
- l) identifying the cells on said first substrate expressing said ligand binding protein of interest.
40.        The method of claim 39, comprising repeating a) through j) at least once.
41.        The method of claim 39, further comprising isolating cells identified in l); growing said cells under conditions that allow for expression of said ligand binding protein of interest; and determining the expression of said ligand binding protein of interest by an enzyme-linked immunosorbent assay (ELISA).